Supporting Information for:

Sunlight-Activated Propidium Monoazide (PMA) Pretreatment for Differentiation of Viable and Dead Bacteria by Quantitative Real-Time PCR

Xing Xie [†], Siwen Wang [†], Sunny C. Jiang [§], Janina Bahnemann [†], Michael R. Hoffmann [*,†]

[†] Linde+Robinson Laboratories, California Institute of Technology, Pasadena, California 91125, United States.

[§] Department of Civil and Environmental Engineering, University of California, Irvine, CA 92697, United States.

[*]Corresponding Author

Phone: 626 395 4391. Fax: 626 395 2940. E-mail: mrh@caltech.edu.

Detailed Methods for DNA Extraction and qPCR

Bacteria cells were collected from both PMA treated and untreated control samples by centrifugation at 17,000×g for 10 min (Centrifuge 5424, Eppendorf Inc.). Genomic DNA was extracted using the PureLink® Genomic DNA Mini Kit (Thermo Fisher Scientific Inc.) following the manufacturer's instructions. Real-time PCR assays were performed with MasterCycler RealPlex 4 (Eppendorf Inc.) to quantify the presence of universal bacterial 16S rRNA gene. The reaction mixture contains 10 μL PerfeCTa® qPCR ToughMix® (Quanta BioSciences Inc.), 0.25 μM forward primer (1369F, 5′CGGTGAATACGTTCYCGG3′, where Y is either C or T, Integrated DNA Technologies Inc.), 0.25 μM reverse primer (1492R, 5′GGWTACCTTGTTACGACTT3′, where W is either A or T, Integrated DNA Technologies Inc.), 0.25 μM TaqMan probe (FAM-5′CTTGTACACACCGCCCGTC3′, Integrated DNA Technologies Inc.), 1 μL template DNA, and nuclease free water (Promega Corporation) to a final volume of 20 μL.[1] For the thermal cycling, the initialization was 3 min at 95 ^{o}C, followed by 40 cycles of 15 s at 95 ^{o}C for denaturation and 30 s at 55 ^{o}C for annealing/extension.

Supplementary Figures

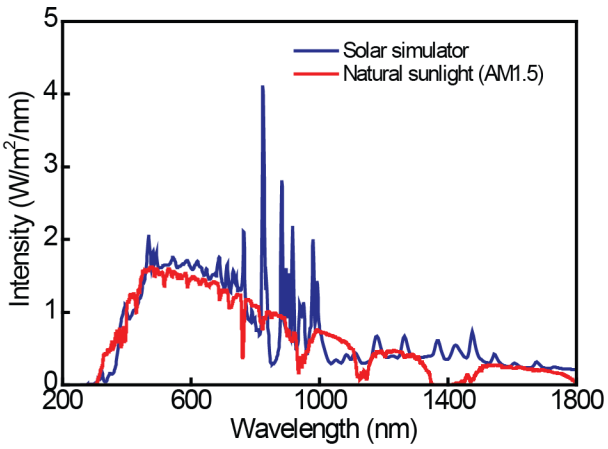

Fig. S1. Spectrum of the solar simulator (provided by vendor: Sun 2000, Abet Technologies Inc.) and natural sunlight at AM1.5.[2]

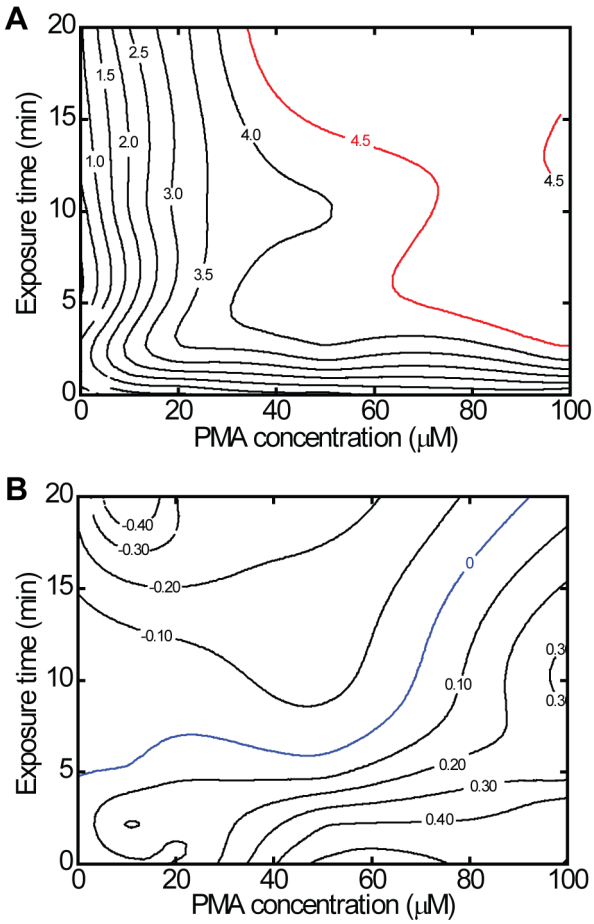

Fig. S2. Signal reduction (ΔC_t values) in qPCR assays when samples containing dead **(A)** or viable **(B)** *E. coli* cells were pretreated with different PMA concentrations (10, 20, 50, 100 μM) and times of sunlight exposure (1, 2, 5, 10, 20 min). The ΔC_t values, calculated by subtracting C_t values of PMA untreated samples from that of treated samples, are represented by the contour lines generated using OriginPro.

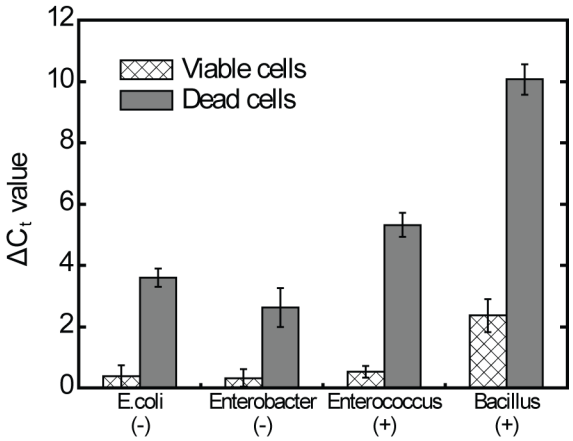

Fig. S3. Signal reduction (ΔC_t values) in qPCR assays when samples containing various bacterial cells were pretreated with sunlight-activated PMA (80 μM, 10 min). The ΔC_t values were calculated by subtracting C_t values of PMA untreated samples from that of treated samples.

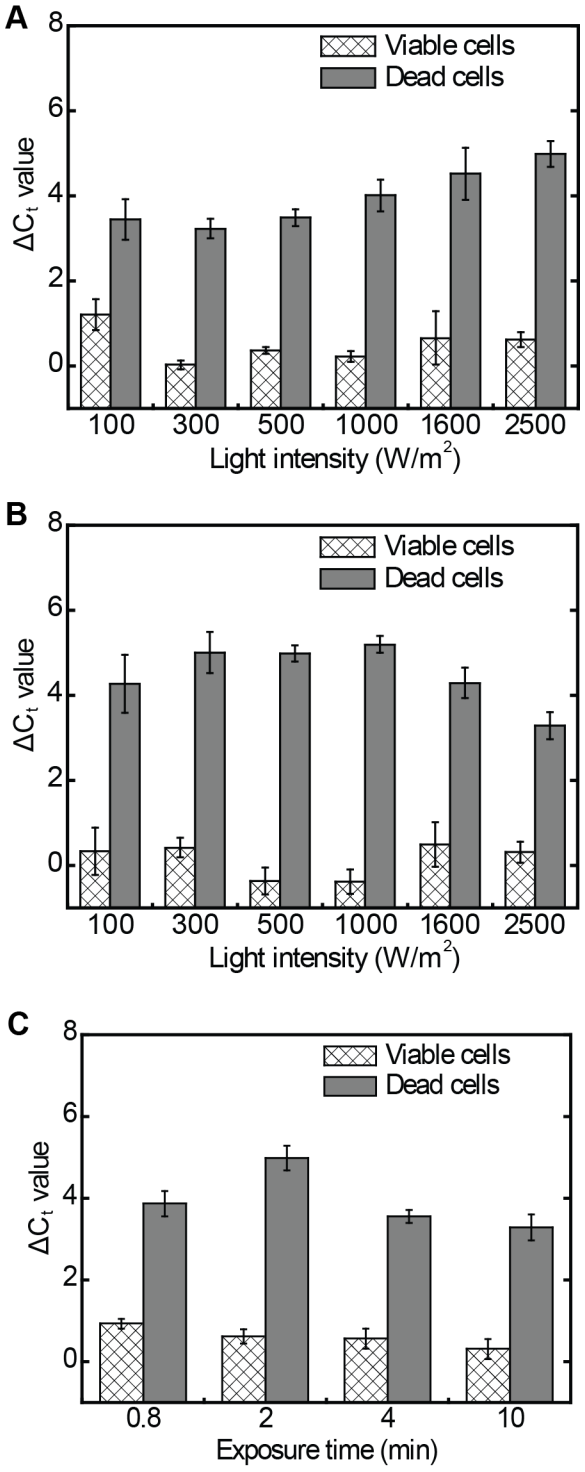

Fig. S4. Effect of sunlight intensity and exposure time on the signal reduction (ΔC_t values) in PMA-qPCR assays. Bacterial samples containing ~1.0×10^9 CFU/mL *E. coli* were pretreated with 80 μM PMA. **(A)** Exposure time was fixed at 2 min. **(B)** Exposure time was fixed at 10 min. **(C)** Light intensity was fixed at 2500 W/m^2.

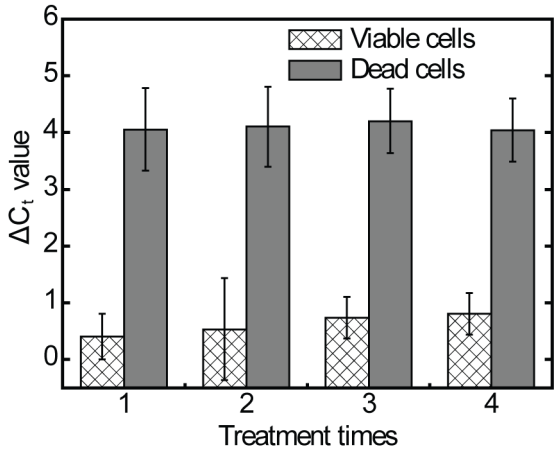

Fig. S5. Effect of multiple sequential treatments on the signal reduction (ΔC_t values) in PMA-qPCR assays. Bacterial samples contained ~1.0×10^9 CFU/mL *E. coli*. All experiment sets had the same total PMA dose (80 μM), incubation time (10 min) and exposure time (10 min). For example, when 4 pretreatments were applied, 20 μM PMA was added each time, and the incubation time and exposure time were both reduced to 2.5 min.

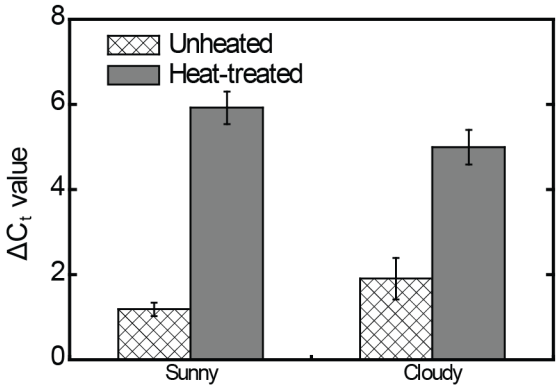

Fig. S6. Signal reduction (ΔC_t values) in qPCR assays when toilet wastewater samples were pretreated with sunlight-activated PMA (80 μM, 10 min). The ΔC_t values were calculated by subtracting C_t values of PMA untreated samples from that of treated samples. The light intensities were 973±6 W/m^2 on the sunny day and 70±10 W/m^2 on the cloudy day.

Supplementary Table

Table S1 Bacterial species used and growth conditions

Species (Gram stain)	Media	T (^{o}C)	ATCC No.
Escherichia coli (G^-)	Fisher BioReagents, LB Broth, Miller	37	10798
Enterobacter cloacae (G^-)	BD Difco™, Tryptic Soy Broth	30	700323
Enterococcus durans (G^+)	BD™ Bacto™, Brain Heart Infusion Broth	37	6056
Bacillus subtilis (G^+)	BD Diagnostics, Nutrient Broth	30	6051

References

1. Suzuki, M. T.; Taylor, L. T.; DeLong, E. F., Quantitative Analysis of Small-Subunit rRNA Genes in Mixed Microbial Populations *via* 5'-Nuclease Assays. *Appl. Environ. Microbiol.* **2000,** *66*, 4605-4614.
2. National Renewable Energy Laboratory. http://rredc.nrel.gov/solar/spectra/am1.5/ (accessed Dec 2, 2015).